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Potential of hyperspectral imaging and multivariate analysis for rapid and non-invasive detection of gelatin adulteration in prawn

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ABSTRACT

In this study, the reliability and accuracy of hyperspectral imaging was investigated for detection of gelatin adulteration in prawn. The spectra of prawns were extracted according to the shape information of prawns contained in the hyperspectral images. Least-squares support vector machines (LS-SVM) was used to calibrate the gelatin concentrations of prawn samples with their corresponding spectral data. The combination of uninformation variable elimination (UVE) and successive projections algorithm (SPA) was applied for the first time to select the optimal wavelengths in the hyperspectral image analysis. The UVE–SPA–LS-SVM model led to a coefficient of determination (r_p^2) of 0.965 and was transferred to every pixel in the image for visualizing gelatin in all portions of the prawn. The results demonstrate that hyperspectral imaging has a great potential for detection of gelatin adulteration in prawn.

1. Introduction

Prawn and shrimp are important aquatic products in the world. However, some fraudulent phenomena have increased in China in recent years. Many incidents have been reported that prawns and shrimps were injected with gelatin-like chemicals to increase weight in aquaculture markets. The gelatin was injected into the head and belly of prawns and shrimps to make them weigh more and look plumper when the frozen prawns and shrimps thawed. The gelatin, which reveals itself while some consumers wash their prawns and shrimps, is a gelling agent that derives from animal skin and bones (Binsi et al., 2009; Karim and Bhat, 2009). The shrimps injected with gelatin have similar color to normal prawns, fresh look and weight gain of 20–30% (Shrimps injected, 2012). Unscrupulous wholesalers can drive down the price of prawns and shrimps 1–2 yuan by injecting food-grade gelatin (Plastic shrimp, 2012). Such behaviors seriously infringe upon consumer rights and interests. Moreover, long-time ingestion of gelatin would cause health-hidden troubles (Plastic shrimp, 2012), and the problem could be worse if industrial-use substances are injected (Wang, 2012). At present, the identification of injected gelatin in prawns and shrimps is usually conducted by naked eyes. After cutting of the head of prawn or shrimp, there might be some faint yellow liquid between the head and body for the gelatin-injected prawn/shrimp. However, in most cases, the gelatin is transparent and human cannot discover it by visual sense. In addition, such identification is destructive, laborious and time-consuming, and is a selective examination, which can only evaluates small numbers of samples. With the significant incidence of gelatin-injected prawns and shrimps being sold in the Chinese market, the detection of gelatin adulteration in prawns and shrimps in a rapid and non-destructive way has become critically important to both consumers and industries in public-health and economic terms.

Visible–near-infrared (Vis–NIR) spectroscopy has gained a wide recognition as a rapid, low-cost and non-destructive technique for monitoring quality attributes of agricultural and food products (Chen et al., 2009; Chen and Lei, 2009). However, one disadvantage of spectroscopy technique is that the spectral measurement of prawn may be inaccurate, because the shape of prawn is not round (Wu et al., 2012a). Another disadvantage is that the spectral measurement is not representative for the determination of the injected gelatin in prawn, as different parts of prawn might have diverse values of gelatin. To overcome the abovementioned disadvantages, the spatial information is required to extract spectral information of prawn accurately and generate the distribution map of gelatin in prawn for the adulterant detection.

Recently, hyperspectral imaging also called imaging spectroscopy or imaging spectrometry, has been reported on the rapid

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and non-invasive assessment of quality distribution of food products (Wu and Sun, 2013a, 2013b; He et al., 2013), such as beef (ElMasry et al., 2011; Wu et al., 2013), pork (Barbin et al., 2012), lamb (Kamruzzaman et al., 2011), fish (Wu and Sun, 2013c, 2013d; Wu et al., 2012b), prawn (Wu et al., 2012a), apple (ElMasry et al., 2008), citrus (Gomez-Sanchis et al., 2008), tomato (Polder et al., 2002), wheat (Mahesh et al., 2008) and maize (Del Fiore et al., 2010). By integrating the main advantages of spectroscopy and imaging into one system, hyperspectral imaging can generate a spatial map of spectral variation, resulting in the capability of quantitatively measurement the inherent chemical and physical properties of the specimen as well as their spatial distribution simultaneously (Wu et al., 2013).

The object of this study was to evaluate the potential of using hyperspectral imaging technique in the spectral region of both visible and near infrared (Vis–NIR) spectral region (897–1753 nm) for the gelatin determination of prawns. The regions of interests of prawns were identified using the spatial information within hyperspectral images. The optimal wavelengths were selected to predict the different gelatin content of prawns. At last, the gelatin distribution of prawns was visualized.

2. Materials and methods

2.1. Samples preparation

Live prawns with five batches were obtained from local markets at five different time from May to July, 2012. Gelatin solution was prepared with the concentration of 2% by adding 2 g pure gelatin solid particle into 100 ml distilled water. The volumes of gelatin solution (2% w/v) added in prawns were 1, 2, 3, 4 and 5 ml. The solution was injected evenly throughout the whole body of the prawn. After injection, the gelatin solution formed a semi-solid colloid gel. There were 20 samples obtained from the five batches for each concentration level, resulting in the adulterated prawn set with a total of 100 samples. Another 60 samples from the five batches were prepared as the control set without adding gelatin. As a result, 160 samples were analyzed in this study. All the prawns were store in −18 °C icebox for 2 days. The frozen prawns were then taken out and laid in 20 °C environment for 1.5 h. Each prawn was scanned individually by the online hyperspectral imaging system described below and the acquired hyperspectral cubes were analyzed using image processing and data mining algorithms to generate the gelatin distribution maps within prawns.

2.2. Hyperspectral imaging system and image acquisition

A pushbroom configuration was used in the system to acquire a spatial line of a hypercube in one frame as well as the whole spectral information simultaneously corresponding to each spatial pixel in the line, resulting in the hyperspectral image stored in a band-interleaved-by-line (BIL) format. As the sample is moving along one direction of spatial 2-D dimensions in this configuration, the line scan system is particularly suitable in conveyor belt systems (MacGregor et al., 2007). There are two imaging spectrographs (ImSpectorV10E and ImSpector N17E; Spectral Imaging Ltd., Oulu, Finland) covering the spectral range of 380–1030 and 900–1700 nm, respectively, in this system. The fiber lines were positioned 150 mm above the conveyer belt and the width of each projected light line on the conveyer belt was 10 mm. The exposure time of camera was 28 ms. Details of the system are described in the literature (Wu et al., 2013).

By using the designed laboratory online hyperspectral imaging system, each prawn was placed on the conveyer belt and then moved at a speed of 4.1 mm s^{-1} to give the same spatial shape of objective in the image. The samples on the *X*-axis moved on and the detector did the linear array scanning along the *Y*-axis direction, resulting in the acquisition of hyperspectral images. As a result, hyperspectral image data of 160 samples were obtained. Among them, 100 samples were used for the calibration and the remaining 60 samples were used for prediction. For each prawn sample, two hyperspectral images were acquired, where one was acquired by using the imaging spectrograph of ImSpector V10 covering the spectral range of 380–1030 nm and the other by using the imaging spectrograph of ImSpector N17E covering the spectral range of 900–1700 nm. Because the responses of the CCD detector in two ranges of 380–441 nm for the imaging spectrographs of ImSpector V10 and 874–941 nm for the imaging spectrographs of ImSpector N17E were rather low and the resulting spectral images at these two particular ranges were rather noisy, hypercubes with only the spectral range from 441 to 1030 nm (with 462 bands) of ImSpector V10 and 941–1734 nm (with 236 bands) of ImSpector N17E were used for further developing the calibration models.

2.3. Image pre-processing

2.3.1. Correction of hyperspectral images

The collected data of the hyperspectral imaging system was the detector signal intensity, rather than the actual reflectance spectra. Therefore, besides the hyperspectral cubes of prawn samples, black and white reference images were also acquired to correct the original hyperspectral images (I_0) into a reflectance mode using the following equation:

$$I = \frac{I_0 - B}{W - B} \times 100 \quad (1)$$

where I is the corrected hyperspectral image in a unit of relative reflectance (%); B is the dark image (~0% reflectance) and W is the white reference image (~99.9% reflectance). The white reference image (W) was acquired from a Teflon white surface under the same condition of acquiring hyperspectral images of samples. The black image (B) was acquired when the light source was turned off and the camera lens was completely covered with its opaque cap. The black image can also be used to remove the effect of dark current of the camera sensor. All the corrected images were then used as the basis for subsequent analysis to extract spectral information, effective wavelength selection, adulterant prediction, and visualization purposes.

2.3.2. Image segmentation and spectral data extraction

Image segmentation is an essential spatial preprocessing step for further spectral and feature extraction. Regions of prawn individuals were isolated from the background by using image processing technique with the aid of Matlab 2009a software (The Mathworks, Inc., Natick, MA, USA). The segmentation of hypercubes in Spectral Set I starts by extracting the images at wavelengths of 655, 551 and 449 nm, and setting them into red, green and blue channels respectively to form an RGB image. The obtained RGB image is then converted into a gray level image by using the Matlab function 'rgb2gray' based on a weighted sum function of $0.2989 \times R + 0.5870 \times G + 0.1140 \times B$. The global thresholds, which were obtained using the Matlab function of "graythresh," were different for each hypercube. This step produced a binary image called 'Prawn Mask'. By masking every hyperspectral image with the prawn mask, the target region of each prawn was obtained with the background shown in black. The segmented region was considered as the region of interest (ROI) of the corresponding prawn sample. A similar segmentation process was conducted for hypercubes in Spectral Set II by forming the RGB image based on the images at wavelengths of 1656, 1355 and 1076 nm. On the basis of the identified ROI, the spectrum of each pixel was then